Further Electron Microscope Studies of a Mouse Leukemia Induced by Cell-Free Filtrates*

By ETIENNE de HARVEN,‡ M.D., and CHARLOTTE FRIEND, Ph.D.

(From the Laboratoire de Microscopie Electronique de L'Institut d'Anatomie, Université Libre de Bruxelles, Brussels; and the Section on Viral Oncology, Sloan-Kettering Institute for Cancer Research, New York)

PLATES 381 TO 388

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ABSTRACT

Further electron microscope observations of tissues from Swiss and DBA/2 mice with leukemia transmissible by cell-free filtrates to the adult animals are presented. The cytological characteristics of the leukemic cells, the fine structure of the viruses, and the virus host-cell relationships have been examined. The leukemia virus has an external diameter averaging 870 A, and appears to be formed at the level of cell membranes by a budding process. The viruses are observed most frequently in intercellular spaces, but are also often found within cytoplasmic vacuoles of megakaryocytes. Lead hydroxide staining was applied to the study of the leukemic material. The viruses have been found to have a considerable affinity for this lead salt, only comparable in intensity to the affinity shown by RNP granules for the same chemical.

INTRODUCTION

In a preliminary report (10), "virus-like" particles were described in spleen, bone marrow, and liver of adult Swiss and DBA/2 mice with leukemia induced by cell-free filtrates (11). Particles were found in about one-quarter of the examined specimens from leukemic mice, and were never observed in non-leukemic mice of the same strains. The particles consisted of two concentric spherical shells, the outer shell having a diameter of approximately 780 A. Of the several hypotheses concerning the relationship of these particles to the disease, the one retained as the most probable was that they were the etiologic agent of the leukemia.

The present study was undertaken to elucidate the following questions. First, what are the characteristics of the leukemic cells? Second, what is the morphological character of the virus? Third, is it possible to identify within the infected cells some precursors of the mature virus particle? Recent advances in electron microscope techniques, especially the electron "staining" method described by Watson (16) have greatly facilitated the study of these problems. It is realized that a purely morphological study sets limited targets, and that a more general interpretation can only be envisioned after an integrated biological and morphological study. The particles, however, will be referred to as "viruses" and no longer as "virus-like," since all specimens were checked for infectivity and proved capable of transmitting the disease.

Materials and Methods

The DBA/2 and the Swiss adult mice were infected by the intraperitoneal injection of 0.2 ml. of filtrates of leukemic spleen suspension. The animals were killed from 21 to 78 days later. Specimens of bone marrow, spleen, and liver were collected and fixed as previously described (10) in a 1 per cent osmium tetroxide solution, buffered at pH 7.3 and containing 7 per cent sucrose (7). Embedding was performed in butyl-methyl (9/1 or 8/2) prepolymerized methacrylate, the complete

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‡ Associé du Fonds National de la Recherche Scientifique (Belgium), Laboratoire d'Anatomie Pathologique, Université Libre de Bruxelles, Brussels, Belgium.

polymerization being obtained by exposure to ultraviolet radiation or at 50°C. Sections were made with a Porter-Blum microtome equipped with diamond knives. In many cases, the sections were collected on grids without any supporting film, and were stabilized by a short exposure to carbon evaporation (8). This technique was found reliable for high magnification work. The microscope used throughout the course of this study was a Siemens Elmiskop I, and the direct magnification used ranged from 4,000 to 40,000. For observation at 40,000 direct magnification, the double condenser was used, with a 400 μ illuminating aperture. Micrographs were recorded on ilford N60 photomechanical plates.

In most instances, the sections, collected on uncoated grids, were stained for 30 minutes with a saturated solution of lead hydroxide, according to the technique described by Watson (16). Contamination with lead carbonate (15) was noticeably decreased by operating in a dry, pure nitrogen atmosphere. The sections were rapidly rinsed in twice distilled water, blotted on filter paper, and finally stabilized by evaporation of a light carbon film.

When solid leukemic tumors (12) became available, they were examined by the same technique.

OBSERVATIONS

Ultrastructure of the Leukemic Cells.—Under the electron microscope the leukemic cells in bone marrow and in spleen of the diseased animals are difficult to differentiate from the normal immature cells seen in these organs. In spite of the fact that the proportion of mature blood cells appears decreased in organs of leukemic mice, it is always a problem to ascertain which particular primitive cell participates in the leukemic proliferation and which cell does not. Moreover, the presence of viruses within a cell does not necessarily indicate that this cell is leukemic; megakaryocytes, which apparently do not participate in the leukemic process, are frequently infected with the virus.

There is a better chance of identifying the leukemic cells in the liver. Here, the organ is of a more uniform cellular type; parenchymal and endothelial Kupffer's cells are readily distinguishable and not likely to be confused with other cellular types. In the lumen of the hepatic sinusoids of leukemic mice an abnormally high percentage of white blood cells is seen (Fig. 1). In the cases where the leukemic infiltration of the liver is massive, these cells are seen not only in the lumen of the sinusoids, but also truly infiltrating the liver, that is, displacing parenchymal cells without interposition of endothelial cells. It is assumed that these cells are actively involved in the leukemic proliferation and may be described as typical leukemic cells. They are round or ovoid in shape, and their cytoplasm is relatively poor in cytoplasmic organelles: mitochondria are few, and have a normal structure; the Golgi zone is occasionally enlarged. Ribonucleoprotein (RNP) granules are extremely numerous and are homogeneously distributed throughout the cytoplasmic matrix. Occasionally, however, these RNP granules are associated with paired membranes of the endoplasmic reticulum. The nuclei of the leukemic cells are ovoid in shape, and the chromatin network is evenly distributed within the nuclear area. Nucleoli are frequently large and multiple. These cells resemble undifferentiated hematopoietic cells. Viruses were frequently observed, in the process of budding on the surface of these leukemic cells (Figs. 6, 10, 13). It should be repeated that the leukemic character of these cells was deduced from their abnormal localization, and that these cells do not appear to be basically different from normal primitive cells found in blood-forming organs.

Structure of the Virus.—From the viewpoint of descriptive virology, several questions arise: How frequently are the viruses found, where are they located, and what do they look like?

A total number of 61 specimens have been so far examined. Viruses were observed in 10/23 (43 per cent) spleens, 10/14 (71 per cent) bone marrows, 5/21 (23 per cent) livers, and 3/3 (100 per cent) solid tumors of leukemic animals. The viruses have been observed in three different sites: (*a*) in the intercellular spaces (Figs. 5, 14, 15), (*b*) budding at the surface of leukemic cells (Figs. 6 to 12), and (*c*) within the cytoplasmic vacuoles of megakaryocytes (Figs. 2 to 4).

What the relationship of the virus to the megakaryocytes is remains unclear. The number of megakaryocytes within the bone marrow of leukemic mice is apparently normal. The structure of these cells generally consists of an extremely large and multilobulated nucleus, and a cytoplasm particularly rich in vacuoles, mitochondria, and membranes in its internal zone (Fig. 2). A peripheral layer of the cytoplasm of these cells appears, on the contrary, poor in these organelles and occasionally shows large pseudopodia. The endoplasmic reticulum appears as a net of smooth membranes forming numerous vacuoles of rather uniform size. In infected megakaryocytes, the viruses are within these cytoplasmic vacuoles (Figs. 3, 4, 17). The viruses were never observed

TABLE I

Dimensions in Angstrom Units of the Viruses Observed in the Tissues from Leukemic Mice

	Mean	Range	No. of measurements
Outer shell diameter	870	730–1370	46
Inner shell diameter	520	300–830	49
Outer shell thickness	53	15–110	23
Inner shell thickness	48	34–130	27

in the nuclei, the mitochondria, or in the cytoplasmic matrix.

The number of viruses varies greatly from one specimen to another, and also between different blocks prepared from the same specimen. The largest group observed rarely exceeded 15 or 20 viral units. Smaller groups, or even apparently single viruses, were more usual.

The viruses are "doughnut" shaped and resemble the viral type *A* described by Bernhard (5). They appear as two spherical concentric shells or membranes of equivalent thickness and density under the fixation methods used. In many cases, the space between the outer and the inner shells is more dense than the space within the inner shell. However, the opposite is occasionally observed in particles which correspond to Bernhard's type *C*; in these, the inner part presents the maximum density. Types *A* and *C* were sometimes observed in the same group of viruses (Fig. 5). Both types of viruses were found to be similar in size. The measurements are listed in Table I, and their range will be discussed later.

After lead hydroxide staining, the contrast of the virus is greatly enhanced and the recognition of isolated viruses becomes much easier. The thickness of the virus shells is not modified by the lead hydroxide staining, but the density is markedly increased and, in many cases, the inner virus shell is particularly well contrasted (Fig. 12). Furthermore, lead-stained viruses seem more complex in structure, a third membrane frequently being observed between the outer and the inner shells (Figs. 9, 10, 13, 15). This third membrane is hardly distinguishable in unstained material and will be provisionally referred to as the intermediate shell of the virus. Its thickness is about 60 A units, and its density is less than that of the two other virus shells.

Although the lead hydroxide staining produces an over-all increase in the contrast of the cellular structures, it should be pointed out that this lead salt has the special affinity for the viruses that it has for RNP granules.

Virus-Host Cell Relationship.—Some aspects of the viruses seem related to their cytologic localization. As already mentioned, megakaryocytes of bone marrows and spleens of leukemic mice frequently contain a large number of viruses within the lumen of cytoplasmic vacuoles. In these vacuoles, the viruses seem better preserved than those lying in the intercellular spaces, their outer membranes being regular and continuous. Moreover, in some cases, the external membranes of several viruses are continuous, forming dumbbell, chainlet, or pyramidal profiles (Figs. 3, 4). Such paired viruses were only observed within the cytoplasmic vacuoles of megakaryocytes.

In many cases the viruses are in intimate contact with cell membranes of leukemic cells, suggesting that the virus particle is formed at the level of the cell membranes by a budding process. The first observable step is a slight bulge in the cell membrane, which increases in density and thickness at this point (Figs. 6, 7). The underlying cytoplasm appears normal and shows an abundance of RNP granules, mostly free in the cytoplasmic matrix. At this step, about 300 A under the bulging membrane, a second membrane begins to form with the same curvature but definitely more dense (Fig. 6). The next step is the transformation of the bulge into a true bud, clearly recognizable as the future virus particle (Figs. 7 to 9). The budding membrane forms approximately half the outer virus shell, and the second membrane may be identified as the future inner shell. The third step is characterized by the completion of the inner shell (Fig. 12), the outer one being still open and participating in the formation of a short pedicle attaching the virus to the cell (Figs. 10 to 12). Narrowing and rupture of this pedicle apparently liberates the mature virus into the intercellular spaces. Budding viruses, or viruses still attached by their pedicle, always present maximum density between the outer and the inner shell (type *A*). Since the type *C* viruses have been observed only in the intercellular spaces, and never in the process of budding, this may indicate that they are probably further along in the cycle. Lead hydroxide staining also facilitates the detection of an intermediate membrane of the virus from the early steps of the differentiation process.

Since cells with buds are presumed to be involved in the process of virus development, it was of interest to search for a more primitive component of the virus within these same cells. Thus far, such virus precursors have not been identified. However, in a few cases of virus-producing cells the Golgi zone appears to be abnormally large and rich in numerous regular vesicles. These vesicles do not have the special affinity for lead hydroxide that the viral shells have.

Occasionally, deformed virus particles have been observed in the intercellular spaces. Viruses having the irregular outer shell illustrated in Fig. 5 may have been damaged. Incompletely formed virus, with both shells appearing discontinuous (Fig. 14), may possibly be the result of the premature rupture of its connection with the cellular membrane. Such abnormally shaped particles will be referred to as defective viruses and will be discussed later.

The Solid Leukemic Tumor.—The number of solid tumor specimens examined has been limited, but so far this material has been found to be particularly rich in viruses. This tumor consists of uniform leukemic cells with large nuclei and dense cytoplasm, the density being due to great numbers of RNP granules. In the intercellular spaces, delineated by sinuous or even villous cell membranes, numerous viruses are observed which are similar in structure to those observed in other leukemic tissues. The same budding process occurs on the surface of the solid tumor cells (Figs. 11, 12).

DISCUSSION

The virus observed in the leukemic material under study is considered to be the etiological factor responsible for the induction of the leukemia in the mice (10, 11). The occurrence of virus within megakaryocytes presents an intriguing problem, for it shows that the particles are not limited to leukemic cells. Since the megakaryocytes do not apparently participate in the leukemic proliferation, there is a remote possibility that the megakaryocytes are infected with another as yet unidentified virus. If, however, virus morphology is considered, the viruses in megakaryocytes and in leukemic cells are strikingly similar, varying only in that viruses enclosed in the same continuous outer membrane are observed only in megakaryocytes. This could be related to special conditions of maturation and does not seem sufficient evidence to discard the hypothesis that the viruses in megakaryocytes are the same as those found in leukemic cells. The localization of the viruses in megakaryocytes might be secondary to a phagocytic process, but this hypothesis is unlikely for two reasons. First, the megakaryocytes are not known to be phagocytic cells (6). Second, the budding process of viruses is seen not only at the surface of leukemic cells, but also along the membranes limiting the cytoplasmic vacuoles of the megakaryocytes (Fig. 17), indicating that the viruses present in these vacuoles differentiate locally from the infected cytoplasm of the megakaryocyte. The membranes limiting these vacuoles are assumed to be part of the endoplasmic reticulum. The fact that the same budding process occurs both at the level of cell membranes and at the level of the limiting membranes of the vacuoles indicates once more how closely related the endoplasmic reticulum and the cell membrane seem to be.

As far as the budding process is concerned, the sequence of steps was tentatively reconstructed as taking a direction toward maturation because the opposite direction seemed improbable. The pictures could hardly illustrate an entrance phenomenon corresponding to the first step of cell infection, because the budding was also observed in vacuoles of megakaryocytes. The budding illustrates a transitional phase in the virus cycle, leading to the mature infective particle previously observed in infective pellets prepared from cell-free filtrates of leukemic tissues (10). An earlier provirus phase has not yet been observed, but it is possible that better resolution or more sensitive staining methods will permit the tracing of the virus throughout its early intracellular developmental cycle.

If the cycle of the virus presently under study is compared to that seen in mouse mammary tumor virus (13), the Golgi zone of the leukemic cells might be suspected of involvement in virus formation (3). In leukemic cells, this zone was sometimes found rich in vesicles. These vesicles, however, do not have the special affinity for lead hydroxide that the virus shells have, and have never been observed near the cell membrane where the process of virus maturation takes place. On rare occasions one single vesicle was seen just behind a budding virus. Although a similar observation was made in the study of chicken erythroblastosis virus (2), more information is needed before any interpretation can be made.

During the first steps of the budding process,

the inner virus shell forms progressively *in situ* and does not correspond, therefore, to a preformed vesicular structure moving from a deeper portion of the cytoplasm. Both outer and inner virus shells are differentiated at the level of the cell membrane. In comparison with the extracellular mouse mammary virus which has four concentric membranes, the leukemic virus has only two. Further, the lead hydroxide staining does not demonstrate a supplementary membrane in the mammary virus (9) as it does in the leukemia virus. The virus of Gross's leukemia (4) appears to be morphologically similar to the one described in the present paper. The budding phenomenon of viruses along cell membranes has also been described for several other viruses (2, 5, 13, 14). The dumbbell or chainlet profiles observed in the infected magakaryocytes bear resemblance to those described in Ehrlich's ascitic tumor (1).

The study of virus fine structure suggests a few remarks on irregularities occasionally observed in virus membranes. The external virus shell is sometimes considerably deformed (Fig. 5). This occurs especially when the viruses are apparently free in large intercellular spaces, and contrasts with the regular outline of the viruses when budding from leukemic cells or when within cytoplasmic vacuoles of magakaryocytes. The significance of these deformities is still obscure. It is possible that the viruses no longer protected by the cell are exposed to the effect of antibodies or to more direct physical injuries resulting from the fixation and the embedding procedure. As far as the timing of the budding process is concerned, it should be noted that the last step characterized by the formation of the pedicle is seen less often than the earlier steps, and could, therefore, be considered as a rapid phase of the phenomenon. Premature rupture of the pedicle is perhaps responsible for the appearance of defective viruses in the intercellular spaces (Fig. 14). The present paper confirms our preliminary observations. The mean external diameter of the viruses observed in the earlier work was 780 A. A far greater number of specimens have now been examined, and the mean value is somewhat higher, averaging 870 A. The rather large dispersion of the observed values (Table I) may be a natural phenomenon, since the same dispersion was observed in the chicken erythroblastosis virus (2). Further, this natural variability may be exaggerated by the limited precision inherent in electron microscope measurements. In the leukemic material the viruses were not observed in truly intracytoplasmic sites, that is, floating in the cytoplasmic matrix. The most frequently occurring localization of the virus was within intercellular spaces. It should be realized, however, that the intercellular spaces observed in thin sections of fixed material do not necessarily have the same proportions that they have in the living tissue.

Finally, the value of lead hydroxide staining in the study of this leukemic material should be stressed. This staining was found most helpful in the delineation of the virus and made possible the observation of its intermediary layer. As observed by Watson (16), the RNP granules have a strong affinity for this lead salt. The virus membranes, especially the inner one, show a similar affinity for the lead hydroxide. Whether the staining method has cytochemical specificity is as yet unclear. Perhaps staining preceded by digestion with specific enzymes will elucidate the analytical value of lead hydroxide.

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Bibliography

1. Adams, W. R., and Prince, A. M., An electron microscope study of the morphology and distribution of the intracytoplasmic "virus-like" particles of Ehrlich ascites tumor cells, *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 161.
2. Benedetti, E. L., and Bernhard, W., Recherches ultrastructurales sur le virus de la leucémie érythroblastique du poulet, *J. Ultrastruct. Research*, 1958, **1,** 309.
3. Bernhard, W., Electron microscopy of tumor cells and tumor viruses, *Cancer Research*, 1958, **18,** 491.
4. Bernhard, W., and Gross, L., Présence de particules d'aspect virusal dans les tissus tumoraux de souris atteintes de leucémies induites, *Compt. rend. Acad. sc.*, 1959, **248,** 160.
5. Bernhard, W., and Guérin, M., Présence de particules d'aspect virusal dans les tissus tumoraux de souris atteintes de leucémie spontanée, *Comp. rend. Acad. sc.*, 1958, **247,**1802.
6. Bessis, M., Traité de cytologie sanguine, Paris, Masson et Cie, 1954.
7. Caulfield, J. B., Effects of varying the vehicle for OsO_4 in tissue fixation, *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 827.
8. de Harven, E., A new technique for carbon films, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 133.
9. de Harven, E., unpublished observations.

10. de Harven, E., and Friend, C., Electron microscope study of a cell-free induced leukemia of the mouse: a preliminary report, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 151.
11. Friend, C., Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia, *J. Exp. Med.*, 1957, **105,** 307.
12. Friend, C., and Haddad, J., Local tumor formation with transplants of spleen or liver of mice with a virus-induced leukemia, *Proc. Am. Assn. Cancer Research*, 1959, **3,** 21, Abstract.
13. Lasfargues, E. Y., Moore, D. H., Murray, M. R., Haagensen, C. D., and Pollard, E. C., Production of the milk agent in cultures of mouse mammary carcinoma, *J. Biophysic. and Biochem. Cytol.*, 1959, **5,** 93.
14. Morgan, C., Rose, H. M., and Moore, D. H., Structure and development of viruses observed in the electron microscope. III. Influenza virus, *J. Exp. Med.*, 1956, **104,** 171.
15. Peachey, L. D., A device for staining tissue sections for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1959, **5,** 511.
16. Watson, M. L., Staining of tissue sections for electron microscopy with heavy metals. II. Application of solutions containing lead and barium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 727.

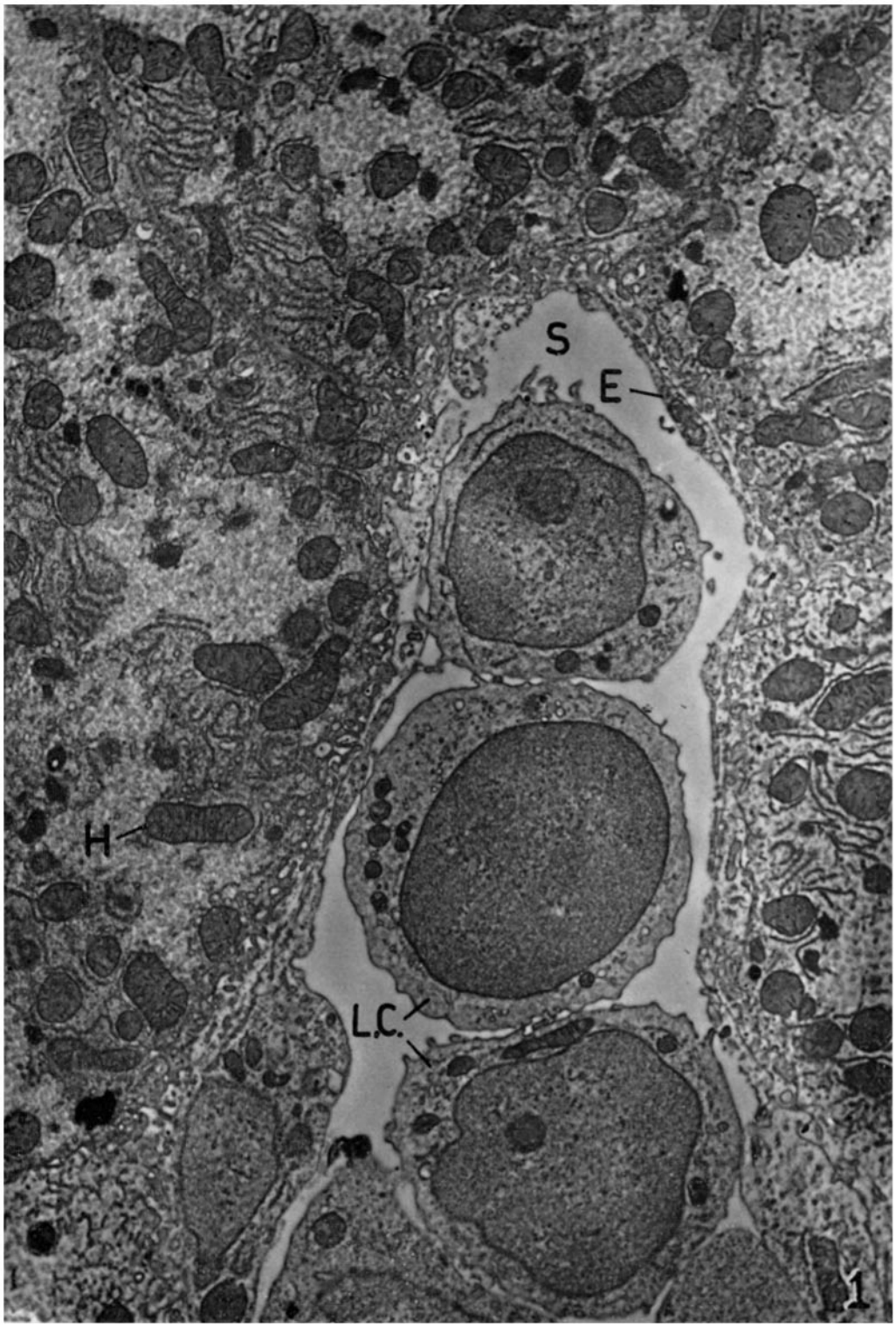
Explanation of Plates

Key to Abbreviation

C, cytoplasmic vacuoles
CM, cell membrane
CP, paired virus forming chainlet profiles
DP, paired virus forming dumbell profiles
E, endothelial cell
H, hepatic mitochondria
I, inner virus shell
L.C., leukemic cell
M, membranes limiting cytoplasmic vacuoles
N, nucleus
O, outer virus shell
P, pedicle bearing a nearly completely formed virus
R, RNP granules
S, lumen of an hepatic sinusoid

Plate 381

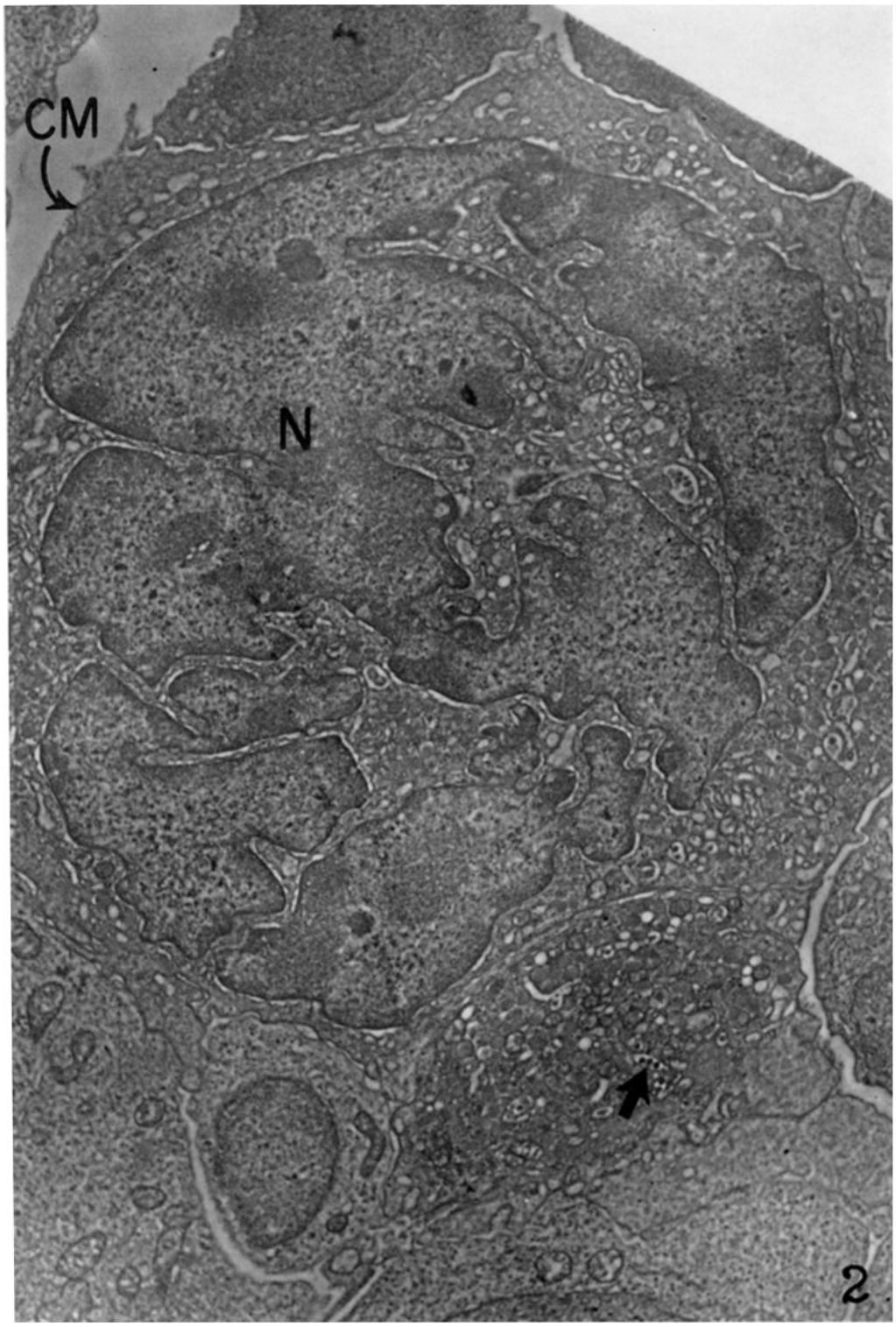
Fig. 1. The liver of a leukemic mouse. Three leukemic cells are seen within the lumen of an hepatic sinusoid. In this case, the sinusoid is limited by a thin sheet of endothelial cells, and the leukemic cells are not truly infiltrating the liver. × 11,000.



(de Harven and Friend: Leukemia induced by cell-free filtrates)

PLATE 382

FIG. 2. The bone marrow of a leukemic mouse. A typical megakaryocyte is characterized by its giant size, its multilobulated nucleus, and its cytoplasm, particularly rich in vacuoles. It is possible to recognize a few dense particles within the lumen of these cytoplasmic vacuoles (arrow). × 11,000.



(de Harven and Friend: Leukemia induced by cell-free filtrates)

PLATE 383

FIG. 3. Portion of the cytoplasm of a megakaryocyte in the bone marrow of a leukemic mouse. The lumina of all the cytoplasmic vacuoles show numerous virus particles recognizable by their regular diameter and their "doughnut" shape. Several viruses are attached to each other, forming dumbbell or chainlet profiles (arrow). One virus (arrow) is in the last step of its differentiation from the membrane limiting the vacuole and is still attached to the cytoplasmic matrix by a narrow pedicle. × 51,500.

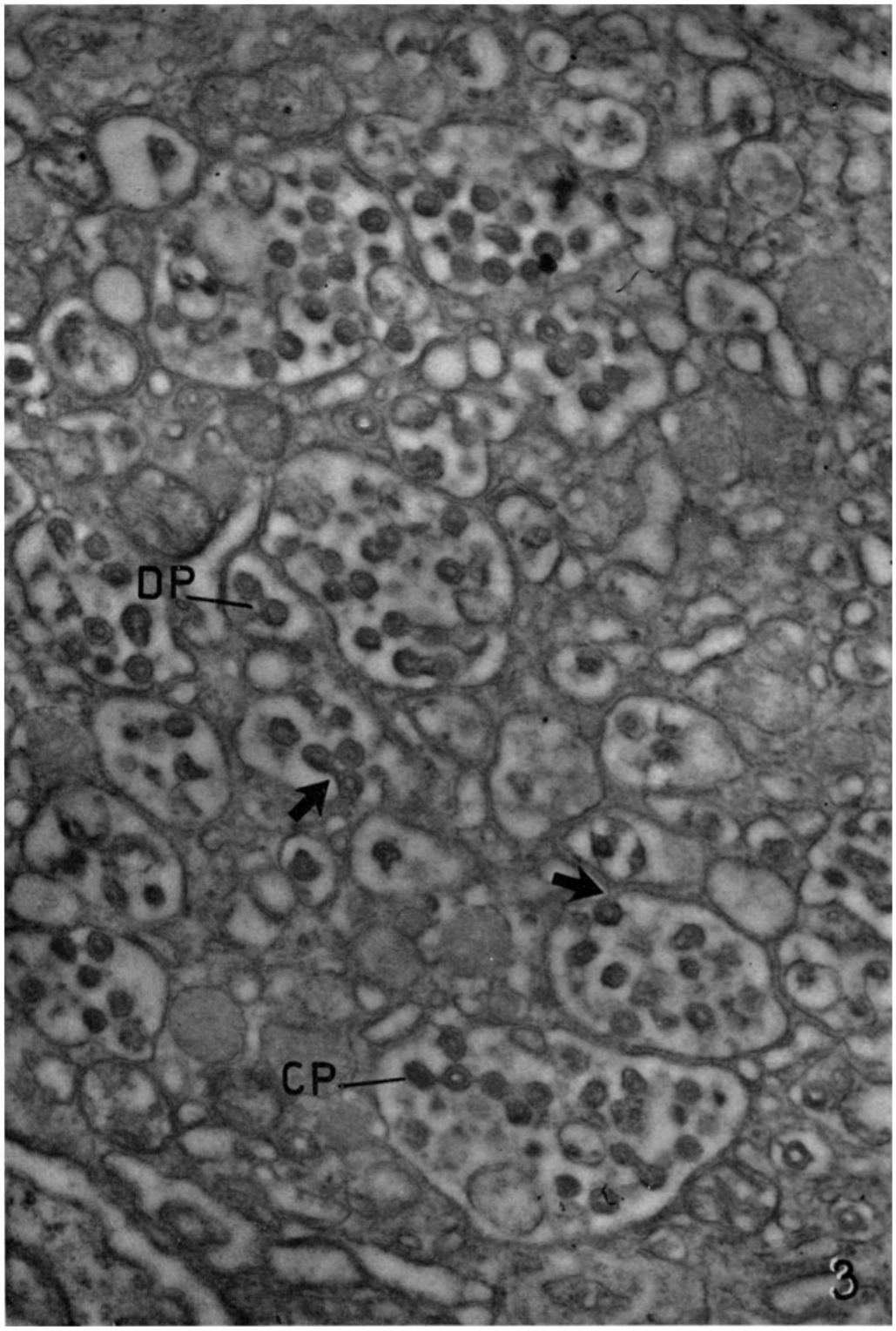


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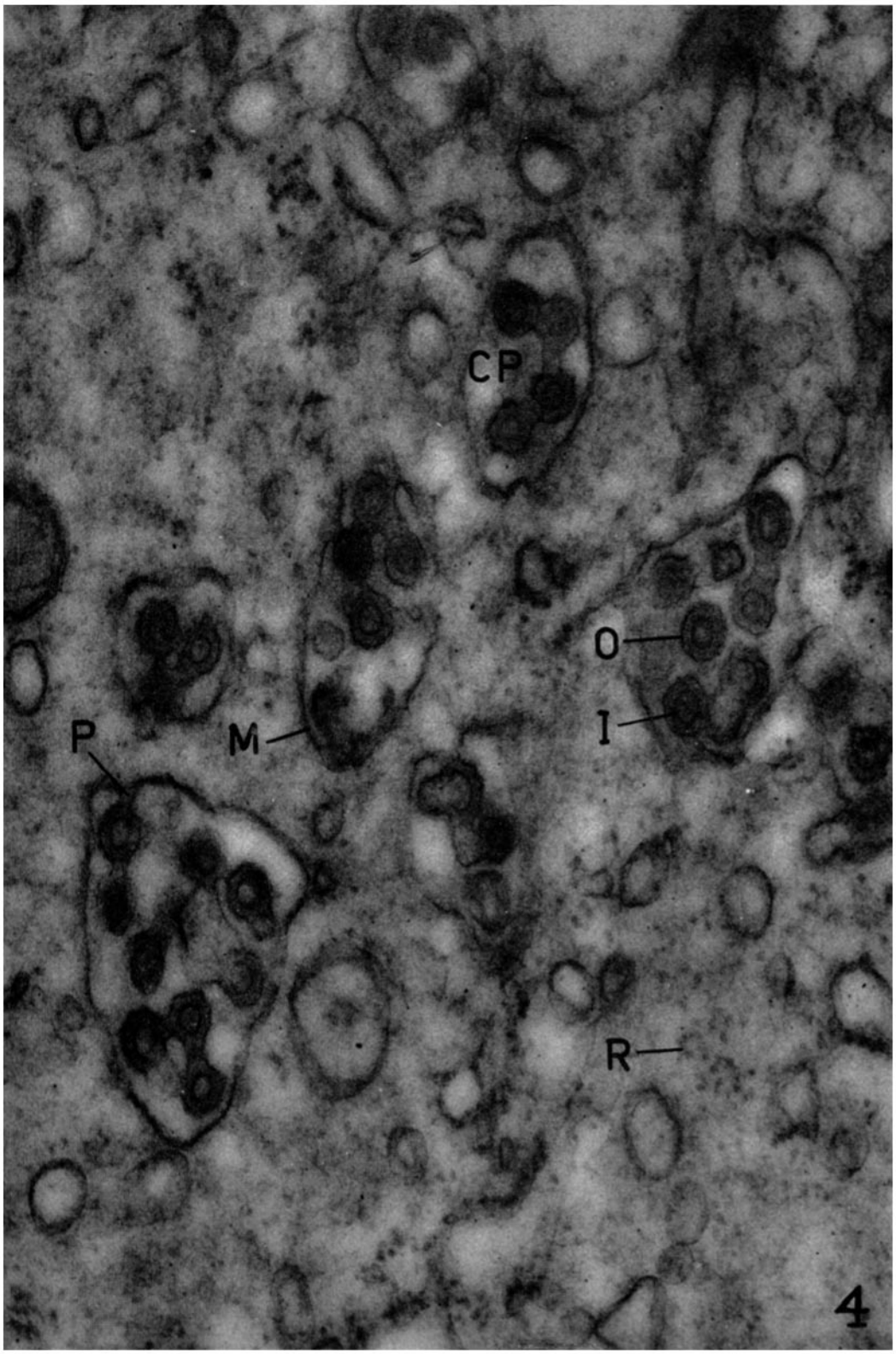
PLATE 384

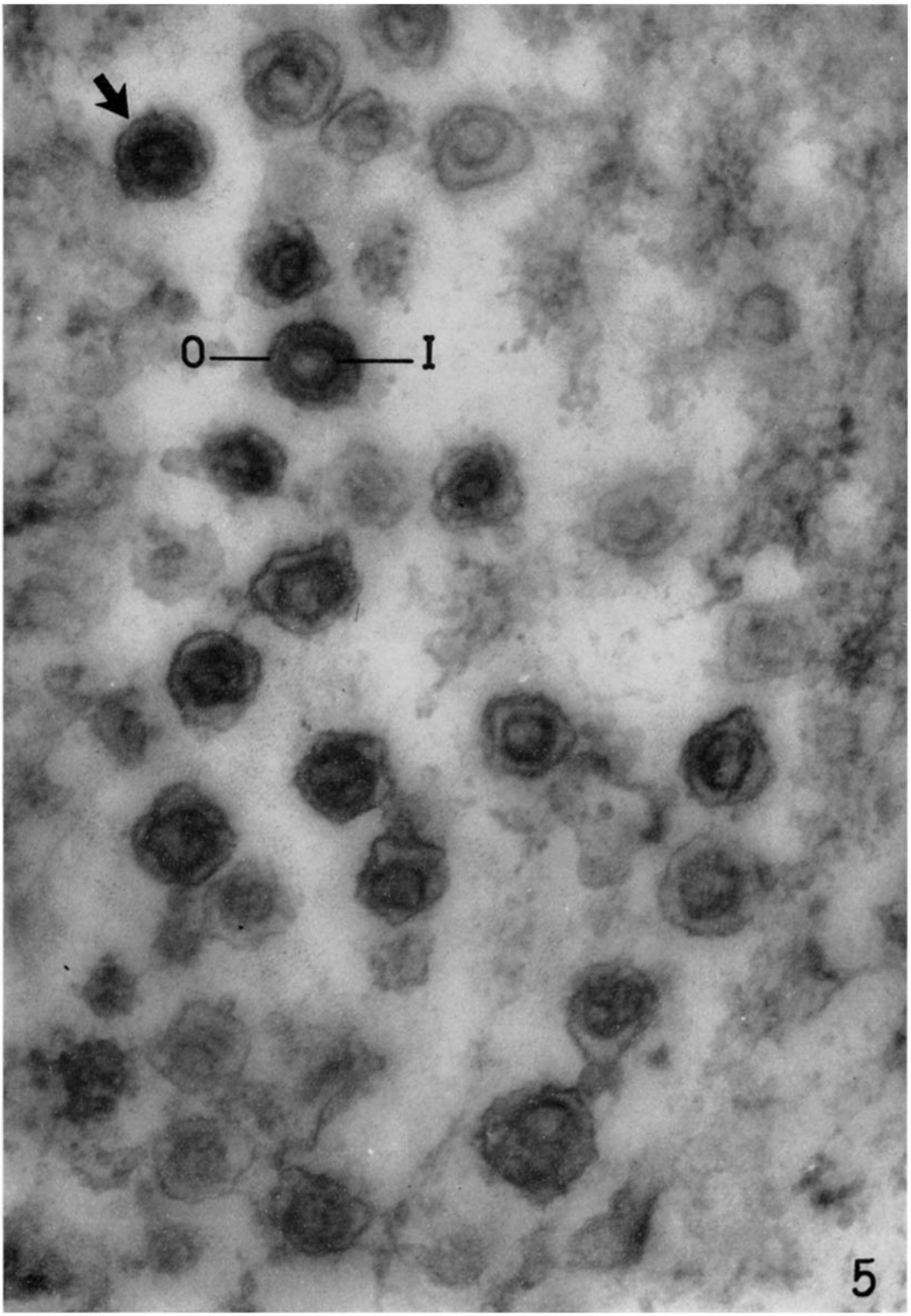
FIG. 4. Portion of the cytoplasm of a megakaryocyte observed in the bone marrow of a leukemic mouse. The structure of the virus consisting of two spherical concentric shells is clearly recognizable. Many viruses are linked together, forming dumbbell or chainlet profiles. The virus shells are regular in outline, and the maximum electron density is observed between the outer and the inner virus shells. All the viruses are within the lumen of cytoplasmic vacuoles. The cytoplasmic matrix shows a few RNP granules. × 81,000.



(de Harven and Friend: Leukemia induced by cell-free filtrates)

PLATE 385

FIG. 5. Several viruses within an intercellular space of the spleen of a leukemic mouse. Lead hydroxide staining. The cell membranes limiting this intercellular space are indistinct, probably because the obliquity of the sectioning or because of polymerization damage. The contrast of the virus membranes is markedly increased by lead hydroxide. Compared to the viruses found within the cytoplasmic vacuoles, these are irregular in shape. In the upper left (arrow) one virus shows its maximum density in the central area. × 168,000.



(de Harven and Friend: Leukemia induced by cell-free filtrates)

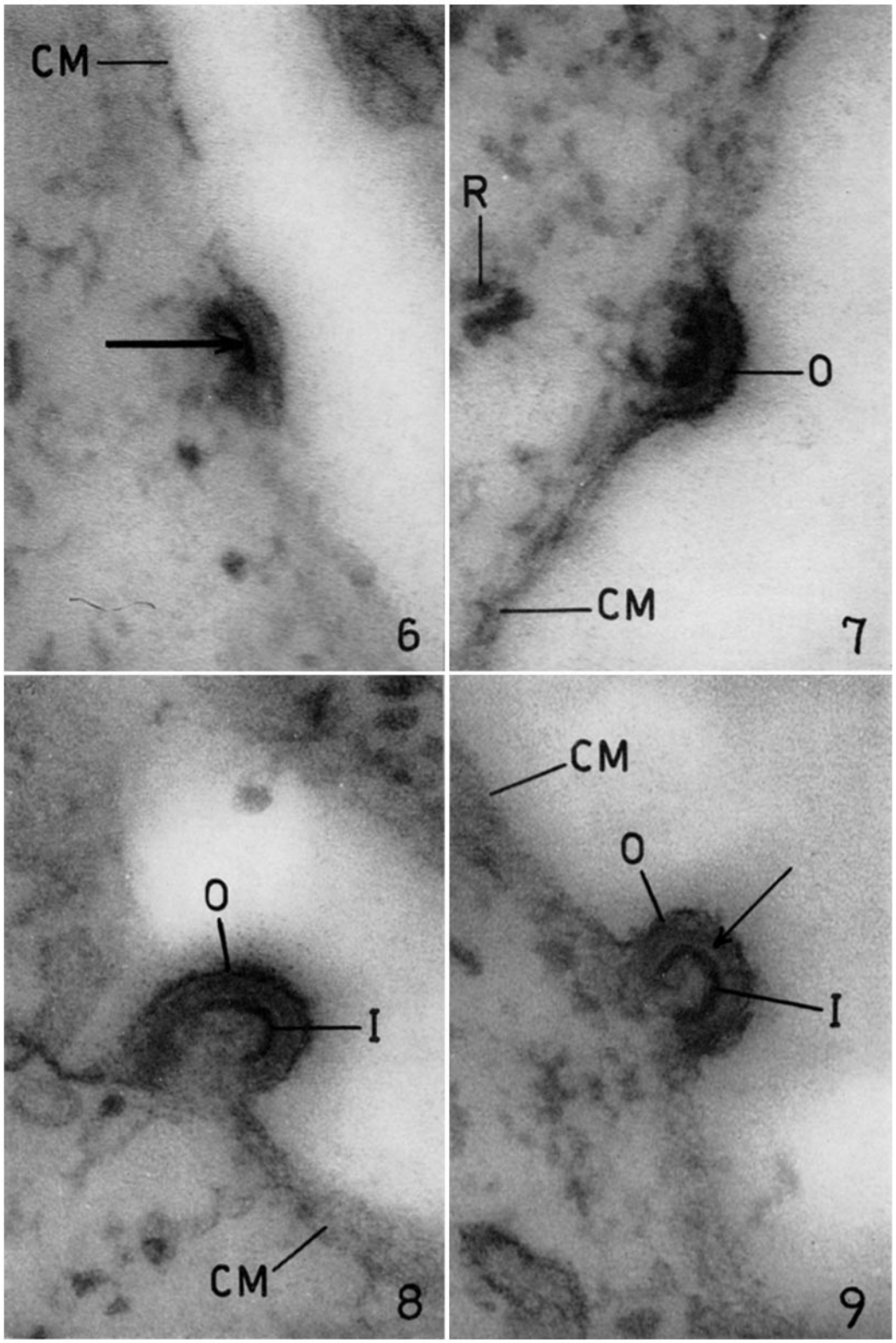
PLATE 386

FIG. 6. Leukemic cells infiltrating the liver of a leukemic mouse. Lead hydroxide staining. A small dense bulge of the cell membrane appears to be the first recognizable step of the virus maturation. The early inner virus shell is seen as a short dense crescent (arrow). × 290,000.

FIG. 7. Spleen of a leukemic mouse. Lead hydroxide staining. Transformation of the bulge into the virus bud. The density of the material participating in the elaboration of the inner virus shell is comparable to that of the RNP granules. × 290,000.

FIG. 8. Bone marrow of a leukemic mouse. Lead hydroxide staining. Virus bud at the level of the cell membrane. The future inner shell is recognizable as is the beginning of the formation of the pedicle. The central part of the virus is filled with a moderately dense granular material. × 290,000.

FIG. 9. Spleen of a leukemic mouse. Lead hydroxide staining. The inner shell is almost completely formed. The intermediate membrane of the virus is also recognizable in this case (arrow). × 290,000.



(de Harven and Friend: Leukemia induced by cell-free filtrates)

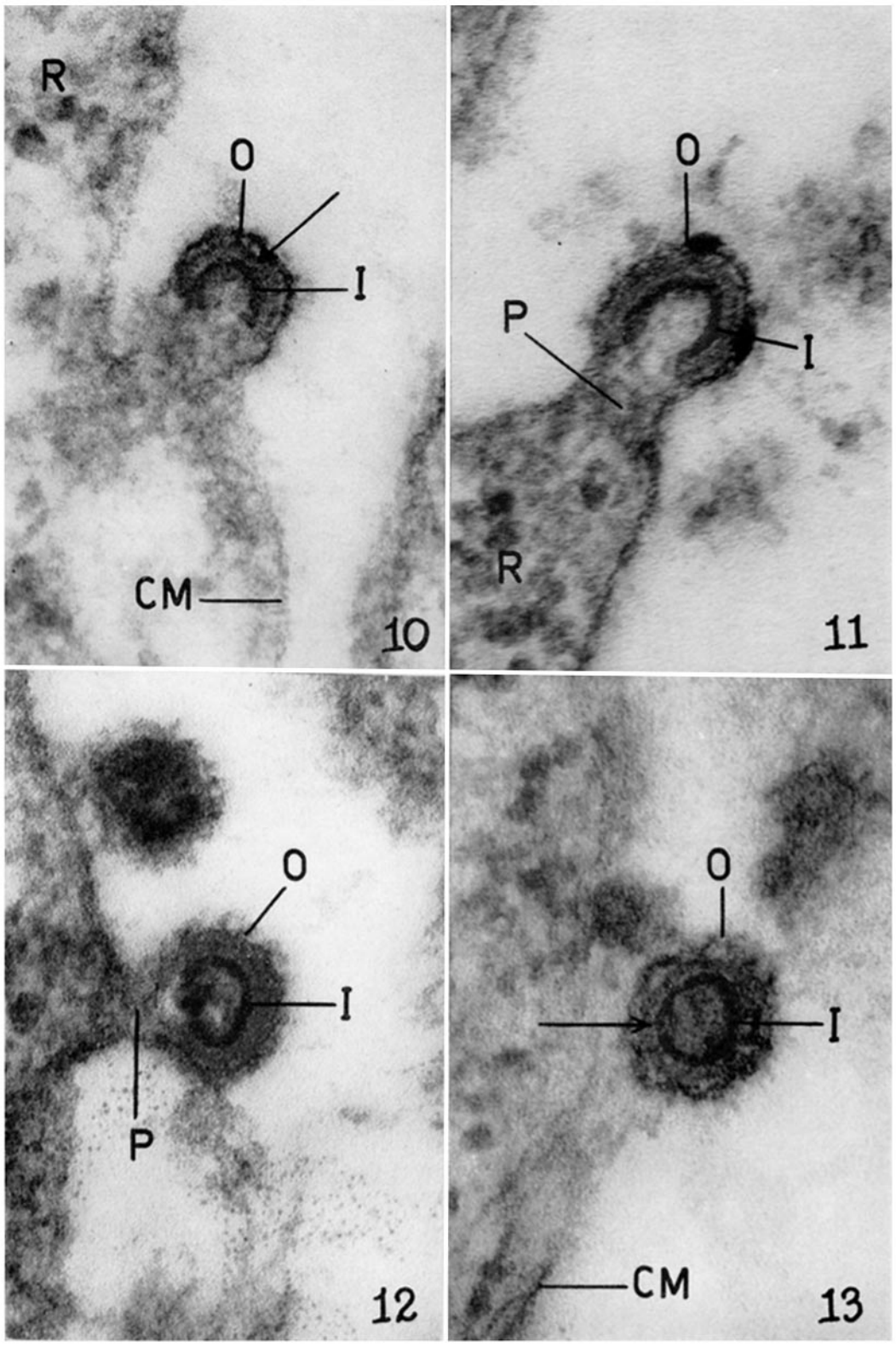
PLATE 387

FIG. 10. Leukemic cell infiltrating the liver. Lead hydroxide staining. Formation of a pedicle bearing an almost completely formed virus. Intermediate membrane can be seen (arrow). × 290,000.

FIG. 11. Same step observed at the level of the cell membrane of a solid tumor cell. Lead hydroxide staining. × 290,000.

FIG. 12. Narrowing of the pedicle observed in a solid tumor cell. Lead hydroxide staining. The inner virus shell is almost formed, and its high affinity for the lead hydroxide allows the observation of the thickness and the density of this virus component. × 290,000.

FIG. 13. Along a leukemic cell infiltrating the liver a mature virus is seen without any clear connection with the cell membrane. The intermediate membrane is seen (arrow). Lead hydroxide staining. × 288,000.



(de Harven and Friend: Leukemia induced by cell-free filtrates)

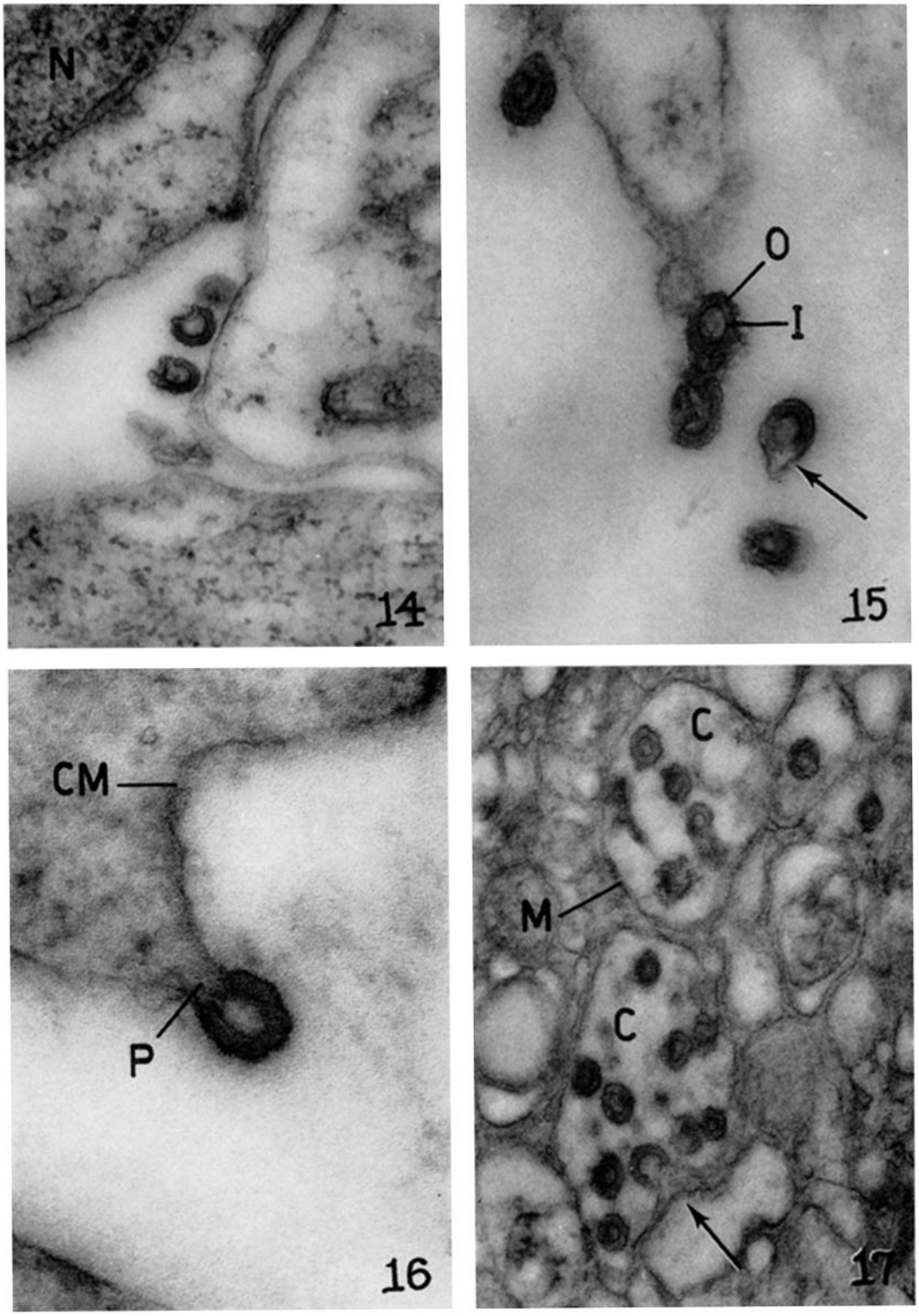
PLATE 388

FIG. 14. Spleen of a leukemic mouse. Lead hydroxide staining. Within the intercellular space two defective viruses are observed, characterized by incompletely formed virus shells. × 100,000.

FIG. 15. Spleen of a leukemic mouse. Lead hydroxide staining. Five viruses within the intercellular space. The virus indicated by arrow may be considered as defective or distorted, and the intermediate membrane is present in this case. × 100,000.

FIG. 16. Bone marrow of a leukemic mouse. Formation of the pedicle of a typical virus bud. No lead staining: the inner virus shell appears poorly contrasted. × 176,000.

FIG. 17. Bone marrow of a leukemic mouse. No lead staining. Viruses are seen within cytoplasmic vacuoles. Arrow indicates a virus bud growing on the membrane limiting the vacuole entirely comparable to the aspects illustrated along cell membranes by Figs. 8 to 10. × 69,000.



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